

MYCOTROPHISM IN THE RUTACEAE.

I. THE MYCORRHIZA OF *ERIOSTEMON CROWEI* F.V.M.

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(Twenty-two Text-figures.)

[Read 23th September, 1932.]

Introduction.

The Rutaceae is a family with which the mycotrophic habit has not so far been associated, but our observations have revealed an endotrophic mycorrhiza in the following species: *Zieria Smithii* Andr., *Eriostemon Crowei* F.v.M., *Eriostemon lanceolatus* Gaertn.* Subsequent examination may indicate that the existence of mycorrhiza in the Rutaceae is more widespread than is suspected and, furthermore, that the mycorrhizal condition will be discovered in other families and genera, in which this habit has not yet been noted. The present communication provides a detailed cytological examination of the mycorrhiza of *Eriostemon Crowei*, but observations made on the other species of the Rutaceae recorded above, indicate a close agreement in detail.

The mycorrhiza of *Eriostemon Crowei* is characterized by arbuscules, sporangioles and vesicles; it therefore falls into the mycorrhizal type which is seen in the majority of herbaceous angiosperms, excluding the Ericaceae and certain specialized groups, and, as Gallaud has recorded, is very widely distributed. This type of mycorrhiza is quite distinct from that of the Orchidaceae, the endophyte of which is characterized by numerous septa, and by the "pelotons" in the mycorrhizal cells. The endophyte of the former mycorrhiza shows probable affinities with the Phycomycetous fungi, that of the latter with the Basidiomycetes.

Occurrence of the Mycorrhiza.

Eriostemon Crowei is a common component of the shrub stratum of the *Eucalyptus piperita*-*E. haemastoma* sclerophyllous forest of the Hawkesbury Sandstone habitats near Sydney. The habitat is typically a loose sandy soil, with low water content (average of three estimations on air-dry soil taken from depth of 5 inches, i.e., area of large number of rootlets, was found to be 0.77%), and also a low humus content. The humus content as determined by ignition was found to be 3.2% at depth of 5 inches, while the average hydrogen-ion concentration was pH 5.7 at the surface of the soil and pH 6 at 5 inch depth. The surface layer of the soil, especially in shady situations under *Eucalyptus* spp. and *Angophora lanceolata*, is often composed of decaying vegetable matter, and

* Since this paper was completed a similar mycotrophic habit has been observed by the authors in two additional genera of the Rutaceae, *Boronia* and *Correa*.

favours fungal growth. The mycorrhiza is typically endophytic, and is practically confined to the young primary fibrous laterals, of which very few do not reveal some degree of infection.

The mycorrhiza was first discovered on the roots of very young seedlings, only two or three inches high, but subsequent examination of larger plants in full flower and fruit, disclosed its presence on their rootlets also. Macroscopically the wholly infected rootlets or infected portions of roots with discontinuous infected areas, can readily be distinguished from uninfected, by their pale yellowish, and somewhat oily appearance. Infection does not extend to the apical meristematic portion of the rootlets. Root hairs are comparatively scanty in development and are quite short.

The old secondarily thickened roots are relatively free from the endophyte; only in very few was any trace of the mycorrhiza seen and in those cases the fungus was in its last stages of digestion. As the primary cortex is discarded during secondary growth, the occasional infections of the secondary cortex are probably made directly from the soil.

That the mycotrophic condition of *Eriostemon Crowei* is not exceptional and local, but typical and widespread, is suggested by the fact that plants from widely separated habitats showed no noticeable variations of the endophyte structurally or developmentally.

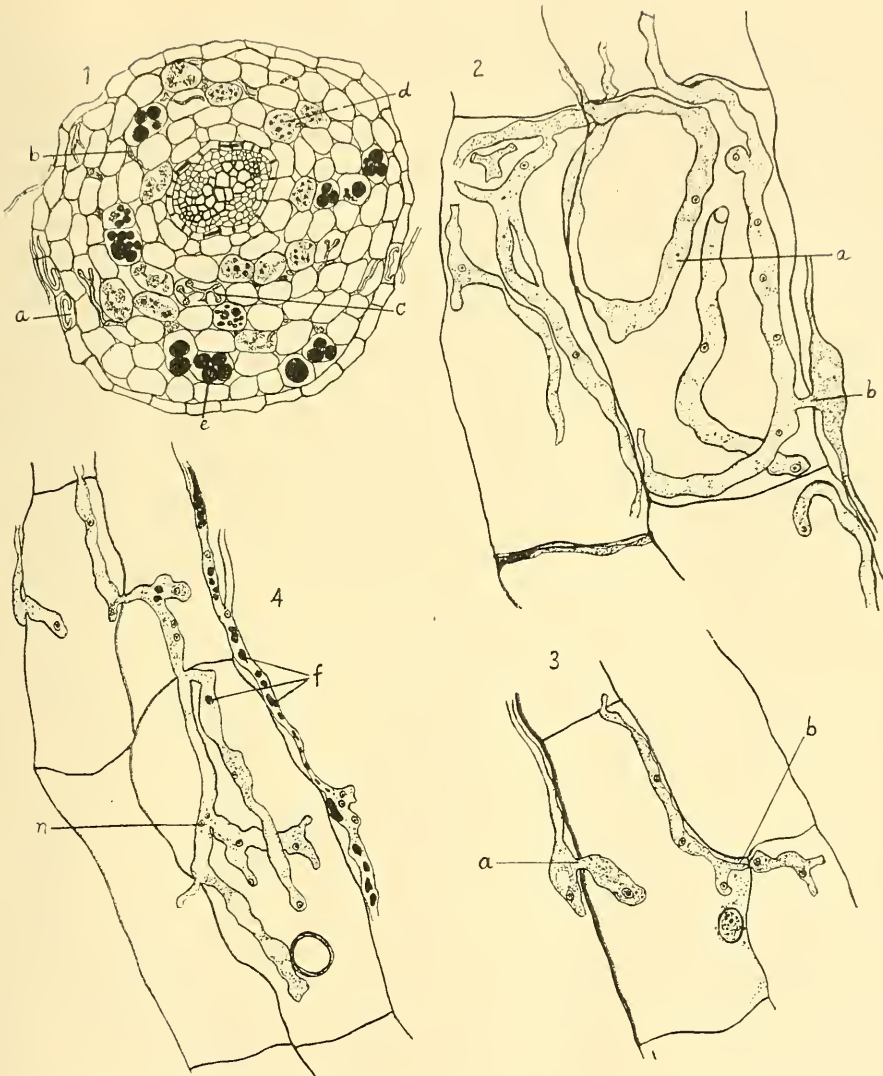
The Root. Text-fig. 1.

Within the epidermis with its scanty root hair development is the cortex, usually four to six rows of large cells in width, with thin walls, intercellular spaces, and scanty cytoplasmic contents represented by the primordial utricles containing the nucleus. The endodermis is characterized by well-developed Casparé strips, while the stele is typically diarch. The endophyte is distributed throughout the epidermis and cortex, but no trace has been seen either in the endodermal cells or within the stele. In the epidermis the fungus is intracellular only, while in the deeper cortical layers the fungus is both intercellular and intracellular, chiefly intercellular. This distribution of the endophyte within the host brings the mycorrhiza of *Eriostemon Crowei* within the first series of Gallaud's classification of endotrophic mycorrhizas, for all other series are entirely intracellular (Gallaud, 1905).

Methods of Examination.

The fixatives employed were strong chromo-acetic solution, or strong Flemming's. In the latter case many of the sections were left unbleached, in order to study the distribution of the fatty substances throughout the endophyte and the host cells. The most suitable sections were 15μ in thickness, for they rendered it possible to trace hyphae for fairly long distances in longitudinal sections of the rootlets. Thinner sections were cut when necessary for more detailed cytological study of the fungus and its vesicles.

The following stains were employed: Safranin and Gentian Violet, Thionin and Orange G (Stoughton, 1930), Pianese III B (Vaughan, 1914), and Haidenhain's Iron-Alum-Haematoxylin. Unbleached osmic-acid preparations were stained in Orange G (saturated solution in absolute alcohol). The Thionin-Orange G combination gave excellent differentiation of the mycelium, but for detailed cytological study of the fungus in all its stages, the Pianese III B and Iron-Alum-Haematoxylin were much superior to any other combinations used by us.



Text-fig. 1.—T.S. of young rootlet showing diarch stele, endodermis, Casparé thickening of radial walls, cortex composed of relatively large parenchyma arranged in 4-6 rows, and epidermis. Fungal mycelium is seen on the surface, also forming coils in the epidermal cells (*a*) and developing both intracellularly and intercellularly within the cortex (*b*), and forming arbuscules and sporangia within the cells (*c*). Disintegrating sporangia are seen at (*d*) and large fat globules staining black with osmic acid at (*e*). $\times 135$.

Text-fig. 2.—Mycelium of the coenocytic endophyte within the epidermis and outer layer of the cortex; the coiling of the hyphae in the epidermis, their wide lumen and sparse contents are showing at (*a*), also the infection-tube formed by the superficial hyphae (*b*). $\times 735$.

Text-fig. 3.—Infection tube penetrating the wall of the epidermal cell, and entering the lumen of the cell at (*a*); a thin-walled hypha is spreading inwards into the cortex at (*b*) and the constrictions of the hypha are shown. $\times 735$.

Text-fig. 4.—Intracellular branching hyphae within an outer cortical cell showing the thin wall, the nuclei (*n*) and the irregular form; further in, fat masses (*f*) are seen in the intercellular hypha. $\times 735$.

Morphology of the Endophyte.

(a). *Extra-epidermal hyphae and penetration* (Text-figures 2, 3, 4, 5).

The fungus forming the mycorrhizal condition in *Eriostemon Crowei* is probably widely distributed in the sandstone habitats around Sydney, and has developed a symbiotic relationship with the roots of certain Rutaceous hosts. Its entrance to the root system is possible through the root-hairs, or through the epidermis directly, or through the germinating seed if infected. Our studies indicate that infection is readily made through the epidermal cells; no infection of root-hairs was observed, while the question of seed infection has not yet been investigated fully. The surface of the rootlets harbours abundant mycelium which is septate, branched and has thick brown walls. Branching is associated with a swelling somewhat resembling an appressorium, which firmly fixes the mycelium to the surface of the root. From these swellings the infecting hyphae are usually developed (Text-figs. 2, b; 3, a). At the point of infection of an epidermal cell the hypha is considerably distended compared with the ordinary distributing hyphae on the root surface (Text-figs. 2, 3). The actual penetration of the walls usually involves a distinct constriction of the hyphae (Text-figs. 3, 4). In order to secure some idea of the frequency of penetration the external hyphae were carefully micro-dissected by means of a fine glass needle mechanically controlled and the short infecting strands recorded. Roots from three different plants were employed. The following table gives the records of these dissections.

Table showing approximate number of penetrations of parts of a Mycorrhizal root.

Plant.	Length of Root.	No. of Penetrations.	Mean per mm. of Root.
A	3mm.	14	4.8
	5mm.	31	
	3mm.	8	
B	4mm.	10	3.4
	4mm.	14	
	5mm.	20	
C	3mm.	9	3.6
	6mm.	15	
	3mm.	12	

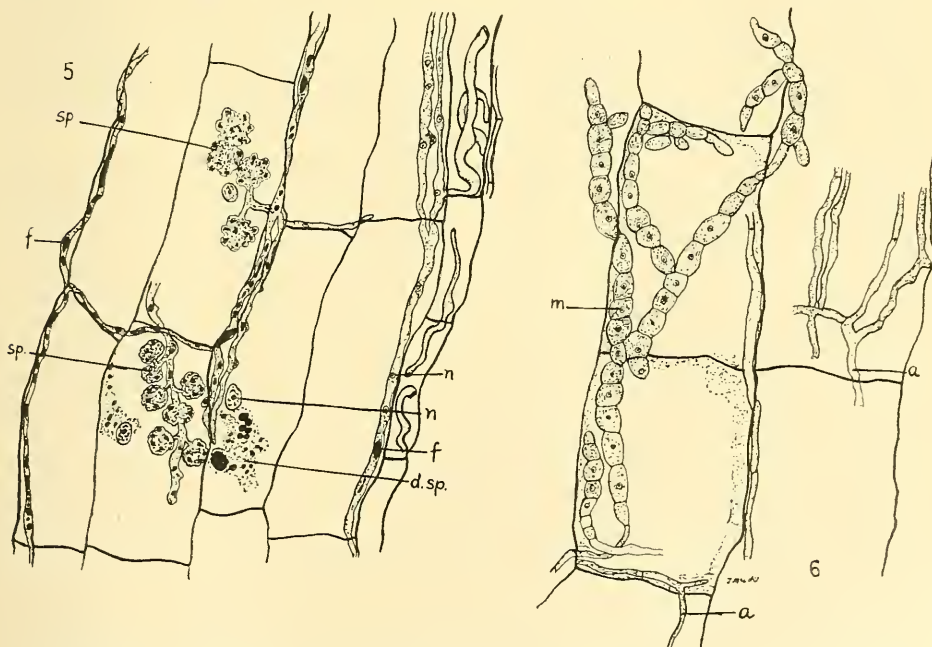
From this table it is indicated that rootlets vary in regard to the frequency of penetration. The mean diameter of the rootlets is approximately 0.37mm., the mean number of penetrations per sq. mm. of surface is 4.1 for A, 2.9 for B, and 2.56 for C. These figures indicate that the internal portion of the fungus is in frequent communication with the soil.

(b). *Epidermal hyphae* (Text-figs. 2, 3, 5).

Once the epidermal cell is penetrated a remarkable change occurs in the fungus, for the endophytic hyphae are non-septate, thin-walled, hyaline, irregular, generally of greater diameter (5-6 μ instead of 2-4 μ), and with little cytoplasm and other cell contents (Text-figs. 2, 3). Within the epidermal cells the hyphae

frequently form an elaborate coil, branches extending from one epidermal cell to another. From the epidermis certain hyphal branches develop inwards to the cortex, and rapidly spread transversely and longitudinally, mainly along intercellular spaces (Text-fig. 5). From one centre of infection the hyphae spread throughout a considerable length of cortex; and while they are usually non-septate, occasional septation appears. The epidermal hyphae not only differ from the internal hyphae in their size, septation, wall structure and cytoplasmic contents, but also probably chemically, for in unstained preparations they are generally yellowish and somewhat waxy in their appearance, and are not readily stainable with those combinations which reveal the contents and cytological details of the more deeply seated hyphae. They are frequently almost devoid of cytoplasm, have few nuclei and little fat, are wholly intracellular, as compared with the intercellular distribution of the cortical hyphae (excepting the arbuscules and sporangioles). They do not spread far from the centre of infection in a vertical direction.

In the mycorrhiza of *Eriostemon Crowei* there is, therefore, a remarkable dimorphism of the hyphae, the extra-epidermal, epidermal and cortical hyphae being quite different from each other, so much so that were continuity of the three forms not determinable, one might be tempted to the conclusion of double infection by different mycelia. Peyronel (1923) who is responsible for this theory of root infection by two distinct fungi was impressed by the dimorphism



Text-fig. 5.—Portion of a longitudinal section of the epidermis and cortex. The coiling of the hyphae within the epidermal cells, the spreading of the cortical hyphae transversely and longitudinally, the young sporangioles (*sp.*), the remains of disintegrated sporangioles (*d.sp.*), fat masses (*f*) and nuclei (*n*) are shown. $\times 300$.

Text-fig. 6.—A portion of the moniloid mycelium (*m*) of a *Rhizoctonia* form in the outer layer of the cortex. Each cell of the moniloid portion is uninucleate; the ordinary hyphae of this endophyte are also shown at (*a*). $\times 735$.

of the mycelium in the mycorrhiza of the Wheat. The dimorphism was in his opinion sufficiently marked to suggest the presence of two independent fungi; and he was convinced that endotrophic infection in the majority of mycotrophic plants is of a composite character, involving two endophytes, one usually less developed—the *Rhizoctonia* type of Orchids, the other more abundantly developed, and with Phycomycete characters. The latter type produces vesicles and arbuscules, the former the characteristic “pelotons” of the Orchidaceous endophyte; Peyronel isolated the Orchidaceous *Rhizoctonia* type of endophyte from *Triticum sativum*, *Zea Mais*, *Hordeum vulgare*, *Arum talicum*, *Beta vulgaris*, etc. The *Rhizoctonia* form from wheat was reinoculated into aseptically seedlings and produced a mycorrhiza with “pelotons”, but without the arbuscules and vesicles usually present in wheat mycorrhizas, and in addition Peyronel found large numbers of vesicles which characterize the phycomycetous form, on dead roots of wheat, maize, etc.

The observations of Mollberg (1884) on *Epipactis* and *Platanthera* describing characteristic “pelotons” in addition to vesicles in the roots; of Gallaud (1915) who described “pelotons” of the Orchidaceous type in the roots of *Tamus* and *Psilotum* along with typical arbuscules; of Petri (1918-1919) who, observing the decay of the mycorrhiza of the Vine and Olive in moist chambers, thought he had secured evidence of the same mycelium producing arbuscules and vesicles within the mycorrhizal cells, yet forming superficial conidial fructifications analogous to those seen in cultures of the Orchid fungi by Burgeff and Bernard, all point to the actual presence of mycelium of two distinct types in the mycorrhizas of some plants. However, these authors attributed the differences in structure and behaviour to a different response of one endophyte to physiological differences in the cells of the root; and concluded that the fungi forming endophytic mycorrhizas all belonged to one group.

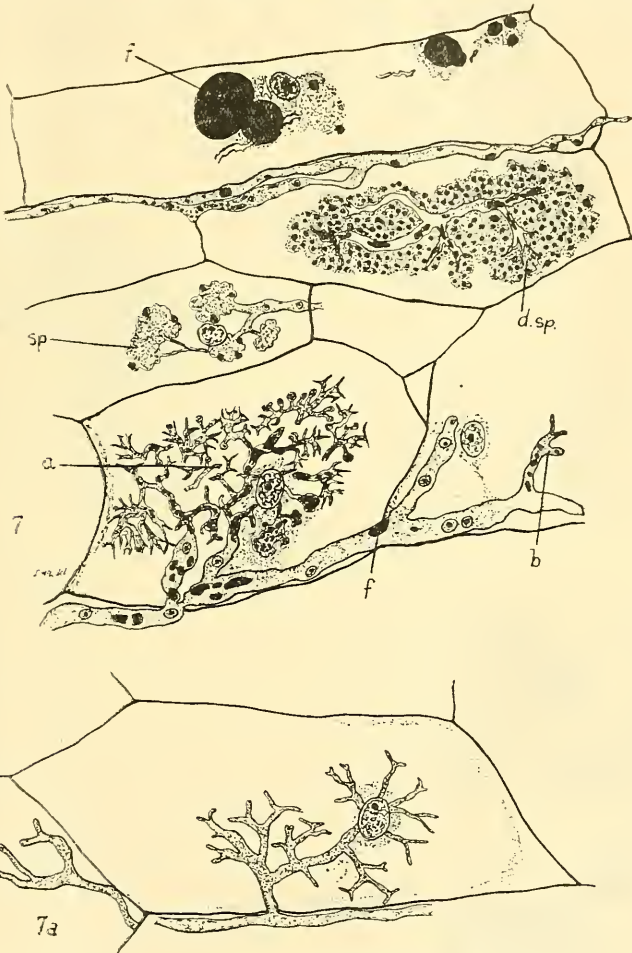
Peyronel, however, seems to have been the first to enunciate explicitly the “double infection hypothesis”, recognizing that some mycorrhizas were formed as the result of infection by two distinct fungi, a primary infection by an arbuscule-forming fungus, and a secondary infection by a *Rhizoctonia* type similar to that of Orchids. Infection by the *Rhizoctonia* form of fungus is confined to the epidermis and outer cortex, and supervenes upon infection by the arbuscule-producing form. Peyronel regards the second endophyte as a quasi-parasite or saprophyte, rather than a true symbiont. It can readily be grown upon artificial media, but the endophytes belonging to the arbuscule-forming group have not been isolated, although a similar mycelium bearing arbuscules and vesicles has been observed in dead roots and in soil, and probably lives saprophytically. In *Eriostemon Crowei* we are able to support Peyronel’s statement of double infection by two distinct fungi, for, in addition to the arbuscule-vesicle-sporangiole forming endophyte which is abundant, especially throughout the entire cortex, and shows the very striking hyphal dimorphism referred to above, there is another endophyte which is confined to the epidermis and outer cortical layer, which develops very characteristic moniliform intracellular hyphae. It is very like the *Rhizoctonia* figured by Peyronel (1924) and isolated from Wheat. Although we have not observed the formation of “pelotons” within the cells, we are convinced that the moniloid mycelium is distinct from the usually coenocytic arbuscule-vesicle forming type which constitutes the endophyte in the mycorrhiza.

In isolation experiments we have obtained a similar *Rhizoctonia* form, but so far have not succeeded in isolating the arbuscule-producing endophyte. Text-figure 6 shows the moniloid mycelium of the second endophyte. While there is

no definite zonation of the endophyte within the cortex itself, we may differentiate the epidermal stage from the cortical owing to the differences discussed above.

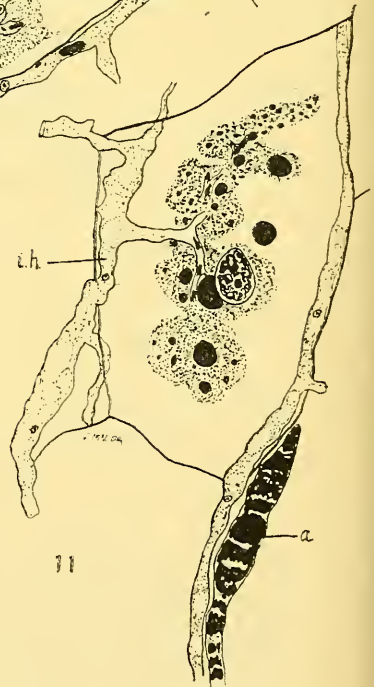
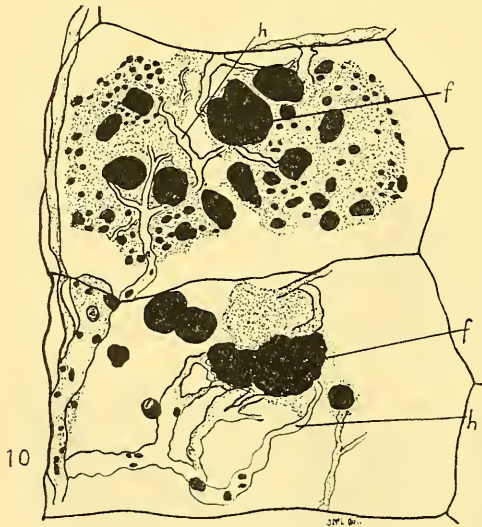
(c). *The Cortical Hyphae* (Text-figs. 4, 5).

Once within the cortex the hyphae spread rapidly both in a transverse and a longitudinal direction for some considerable distance from a centre of infection. The spread of the endophyte within the cortex is mainly intercellular, but it



Text-fig. 7.—Portion of a longitudinal section showing an inter-cellular hypha containing fat (*f*) and forming complex arbuscules (*a*) from lateral branches within the cell. The arbuscules contain dense cytoplasm, nuclei and fat masses, and their ultimate branches are extremely attenuated. A very young arbuscule is seen at (*b*), young sporangioles (*sp.*) with small fat globules and dense cytoplasm, and the residuum of hyphae and fungal cytoplasm and large fat globules (*f*), liberated from the sporangioles are also shown in other cortical cells. $\times 735$.

Text-fig. 7*a*.—A fairly simple type of arbuscule developing from a branch of an intercellular hypha. Note the position of the nucleus of the cortical cell. $\times 735$.



seems capable of readily penetrating cell walls and thus travelling throughout cell cavities (Text-fig. 4). Several hyphae may occupy an intercellular space completely and may somewhat distort the cells in the neighbourhood of the spaces or may even split the longitudinal walls owing to some action upon the middle lamellae. Text-figure 4 shows that the migrating hyphae readily penetrate the walls of the host cells, and are generally constricted at the point of penetration. These cortical hyphae may be designated "distributing hyphae", because they spread the endophyte throughout the cortical region.

The distributing hyphae are thin-walled, of irregular diameter, and frequently simulate an anastomosing structure recalling the laticiferous system of a *Taraxacum*. The cytoplasm is dense, finely granular, with small vacuoles throughout, numerous nuclei with a prominent nucleolus and little chromatic material in the nuclear vacuole, nuclei occasionally in pairs, especially when hyphae are growing rapidly, and variable, but generally large, quantities of fatty material. In some hyphae considerable lengths are stained black with the osmic acid of Flemming's solution (Text-figs. 4, 5), while in others the fatty compounds occur in small rounded or elongated masses. These hyphae lose most of their contents and become practically devoid of fats at the time the arbuscules are developing into the sporangioles.

(d). *The arbuscules and sporangioles* (Text-figs. 7, 7a to 14, 16).

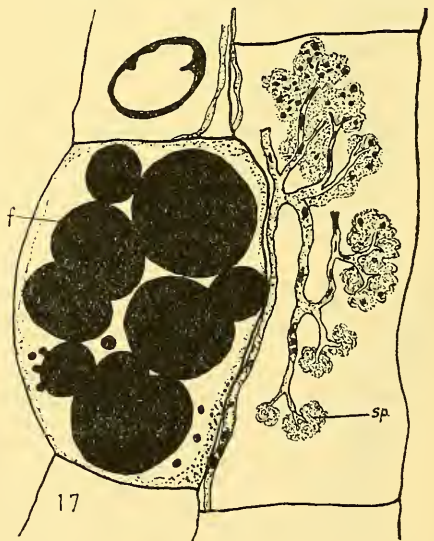
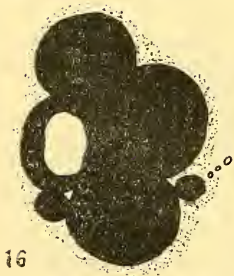
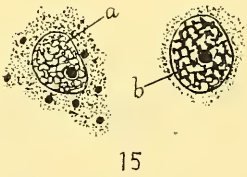
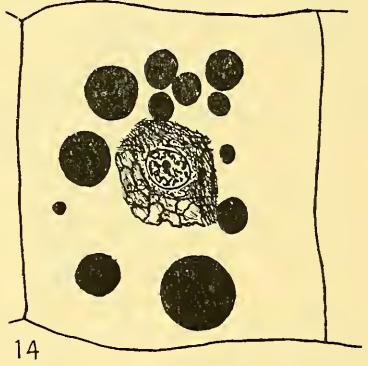
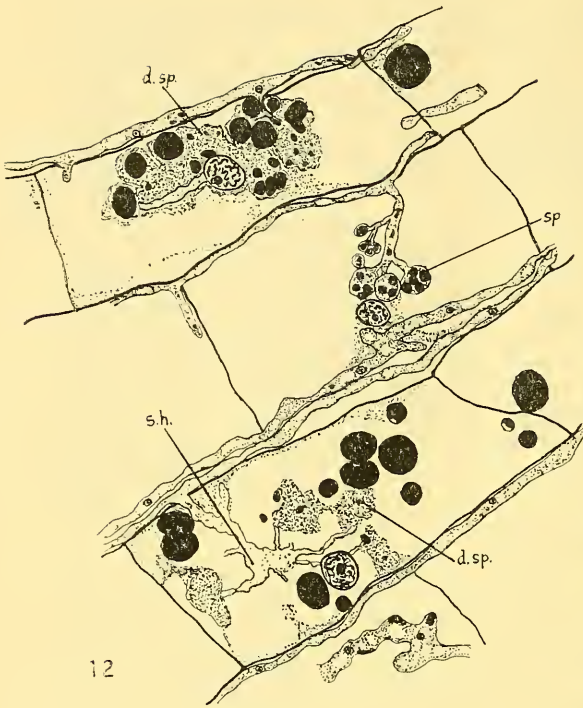
From the intercellular cortical hyphae lateral branches are developed into the cells and produce the characteristic arbuscules and sporangioles. These structures are not confined to any particular region of the cortex as in *Lolium temulentum* investigated by McLennan, but are distributed throughout all the various cell-layers between the epidermis and the endodermis. The arbuscules are not only formed from branches of the intercellular hyphae but also from the intracellular hyphae. In the former case they are usually lateral, in the latter terminal (Text-figs. 7, 7a). In structure the arbuscules are occasionally simple but usually very complex, the early branching being irregular or racemose, the later being regularly dichotomous resulting in a system of very delicate terminal branches (Text-fig. 7). Arbuscules of a very simple type also occur, and several may be developed within one host-cell from a single intercellular hypha only or from an intracellular hypha as well. The arbuscule develops within the cell cavity, and the nucleus, hitherto situated in the primordial utricle, moves towards and takes up a position in close proximity to it. Frequently, the nucleus appears to lie within the complex arbuscule, and at this stage of development the size and chromatic reaction are apparently identical to those of the nucleus of an uninfected host-cell.

Text-fig. 8.—Portion of a tangential section of the middle cortex showing an intercellular hypha which has given rise to several arbuscules of a fairly simple type; the arbuscules are developing the sporangioles (*sp.*), and fat globules (*f*) and cytoplasm are accumulating in them. $\times 735$.

Text-fig. 9.—Cortical cells containing sporangioles which are showing a mamillated structure (*m*) owing to the accumulation of fat globules and cytoplasm within them. In the other cortical cells intracellular hyphae and disintegrating sporangioles (*d.sp.*) are seen; in the latter the membrane which is clearly seen in the young stage is indefinite or dissolved. $\times 735$.

Text-fig. 10.—Cortical cells containing dense sporangioles in various stages of disintegration; the large fat masses are being formed at (*f*). Remnants of the hyphae of the arbuscule are seen at (*h*). $\times 735$.

Text-fig. 11.—Disintegrating sporangioles with fat masses; some hyphae (*i.h.*) devoid of fat while one (*a*) contains an excess. $\times 735$.



The arbuscules at this stage contain dense cytoplasm, nuclei and a considerable quantity of fat, but they rapidly become transformed into sporangioles, apparently by the swelling of the ends of the ultimate branches of the arbuscules. The sporangioles vary in shape and size; some of the very large ones are probably formed by coalescence of a number of small ones. They are usually filled with cytoplasm and fat in their early stage, and appear to have a distinct membrane at first, as if the tips of the finer hyphae had simply become distended by pressure of the accumulating contents (Text-figs. 8, *sp.* and 9); when seen in a mass they appear more or less papillated structures containing cytoplasm and fatty material (Text-fig. 9, *m*). The fatty substances stain black with osmic acid, so that up to this stage there is no micro-chemical change in the fats present in the endophyte. The sporangioles ultimately burst, their membrane becoming very indefinite. Whether this disruption is attributable to the mechanical pressure of the accumulating cytoplasm and fat within them, or whether it is due to some enzymatic action of the host cytoplasm on the sporangiole membrane, one can only surmise. It is noteworthy that in cells with several groups of sporangioles derived from different arbuscules, or different parts of the same one, there is frequently a distinct difference in their development, some being devoid of fat, others abundant in fat, others disrupted and their fat liberated. If the disruption of the sporangioles be caused by the action of a hydrolyzing enzyme produced by the host cytoplasm, then one might reasonably expect the same degree of disintegration throughout, unless there be a time-factor involved in the hydrolysis of the sporangiole membrane or in the development and maturation of the sporangioles themselves.

With the rupture of the sporangioles, their contents, cytoplasm and fat, lie in the cell cavity. The fat globules lie in the mass of fungal cytoplasm derived from the sporangioles and the hyphal branches forming the original arbuscule collapse, and appear colourless and practically empty. From their uniform staining the globules are apparently homogeneous, and are spherical, and still stain black with the osmic acid of Flemming's solution. Text-figs. 9, *d. sp.*, 10, 11 and 12 show the ruptured sporangioles and the liberation of their contents. At this stage the cell-contents are a dense mass of finely granular fungal cytoplasm; numerous fat globules, fragments of shrivelling hyphae, the nucleus and the cytoplasm of the host which can seldom be distinguished from the fungal

Text-fig. 12.—Various stages of the sporangioles and fat accumulation and liberation. Young sporangioles (*sp.*) which are accumulating fat; disintegrating sporangioles (*d.sp.*) which are liberating fat into the cortical cell, and the shrivelling hyphae (*sh.*) of the arbuscules are shown. $\times 735$.

Text-fig. 13.—Mass of cytoplasm (*c*) and fat (*f*) liberated from the sporangioles. Portion of a hypha is also shown at (*h*). $\times 735$.

Text-fig. 14.—Final stage in digestion of the fungal cytoplasm and hyphae within a cortical cell. Note the reticulate residue, the large fat globules and the nucleus of the cortical cells. $\times 735$.

Text-fig. 15.—Nuclei from the midst of a sporangiole complex; (*a*) before liberation of cytoplasm and fat, (*b*) after liberation and during digestion of the fungal cytoplasm. In (*b*) the chromatin appears very much more granular and stains more deeply. The nucleus is much larger than that of an uninfected cortical cell. $\times 735$.

Text-fig. 16, 16a.—Stages in the transformation and translocation of the fat globules from the cortical cells. Finally only a thin dark-staining membrane seems to be left. $\times 735$.

Text-fig. 17.—Portion of a L.S. of the outer cortex of root, showing large fat globules (*f*) in a sub-epidermal cell distending the cell-walls, and sporangioles (*sp.*) at various stages of development, some showing the accumulation of fat, others as yet devoid of it. $\times 735$.

cytoplasm. The outstanding features are the numerous globules and the great amount of fungal cytoplasm (Text-figs. 10, 11). The globules seem to coalesce and form larger ones of different sizes which, in contrast to those described by McLennan (1926) for *Lolium temulentum*, retain their staining capacity with osmic acid. In *Lolium*, McLennan records that the globules are positive to osmic acid while in the hyphae of the arbuscules, but in the sporangioles and when liberated into the cell cavity, they lose this staining property, so that some chemical change has taken place in them. In *Eriostemon Crowei* this change in micro-chemical reaction takes place at a much later period, usually after the digestion of the hyphal mass and fungal cytoplasm in the host cell is well advanced. The fat content of the cell reaches its maximum when all the sporangioles have liberated their contents, and appears to remain constant during the disintegration and digestion of the hyphae of the arbuscules and the fungal cytoplasm, so that there is no discharge of fats excepting through the sporangioles within a cell.

All stages from the developing arbuscules, through the young sporangioles, the bursting sporangioles, to the coalescence of the small fat globules to form a few large ones, and the digestion of the hyphae and fungal cytoplasm, may be seen throughout the cortical cells of a single rootlet in longitudinal section (Text-fig. 12), but there is no characteristic zonation of these phenomena, as described for *Lolium* roots. In each rootlet the endophyte passes through the same stages of development; the rootlet may die or undergo secondary thickening; in both cases any living portion of the endophyte is relegated to the soil; young lateral roots are readily infected during their early growth from the parent root from which they arise.

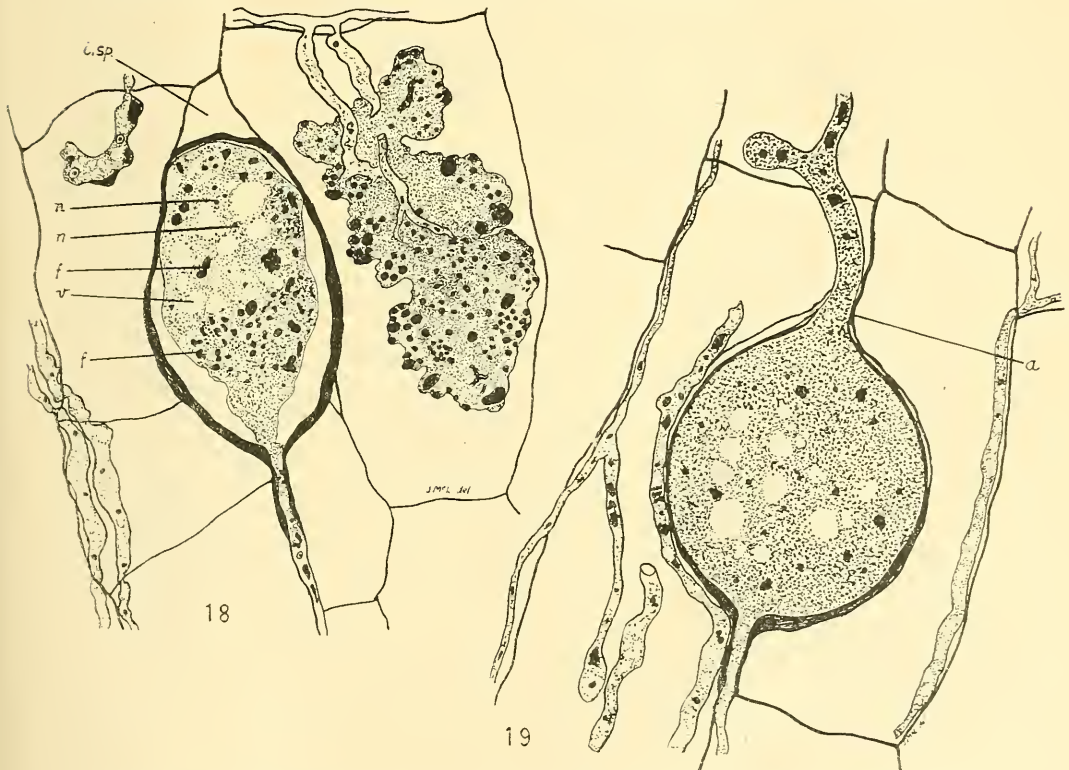
Within the mass of extremely granular cytoplasm and disintegrating hyphae lies the nucleus of the host cell (Text-fig. 11), which by now has undergone a marked transformation. It has increased greatly in size, but does not show any metamorphosis such as the lobing commonly seen in Orchidaceous mycorrhizas; it remains typically ovoid and there is no indication of fragmentation as recorded in other mycorrhizas. The nucleolus undergoes enlargement and there is a great increase in chromatic material which becomes dense, granular, and stains deeply. At this stage the nucleus resembles one in the early prophase of mitosis (Text-figs. 11, 15). The following measurements show the difference in size between the nuclei of infected and uninfected cells.

	Av. of 100 nuclei.	
	Infected Cells.	Uninfected cells.
Length	8.7 μ	5.5 μ
Breadth	7.0 μ	4.6 μ

The length of nuclei of uninfected cells ranges from 7.4 μ to 3.7 μ , the breadth from 6.7 μ to 3.7 μ ; for infected cells the respective ranges are 18.5 μ to 4.5 μ and 9.25 μ to 4.5 μ . These data reveal an average increase of 58% in length and 52% in breadth in the nuclei of infected cells. As this results in a very considerable increase of nuclear volume, and as the chromatic material becomes more abundant, it is apparent that during the processes of liberation of the fat

globules from the sporangioles, and the digestion of the fungal hyphae in the host cells, the nucleus displays an interesting growth phase, which may be the direct result of either abnormal nutrition (as compared with an uninfected cell) or of chemical stimulation. The chemical processes involved in the transformations of the endophyte must involve intense physiological activity within the cell and are partly expressed as nuclear growth. Probably both factors, abnormal nutrition and chemical stimulation, are responsible for these nuclear phenomena.

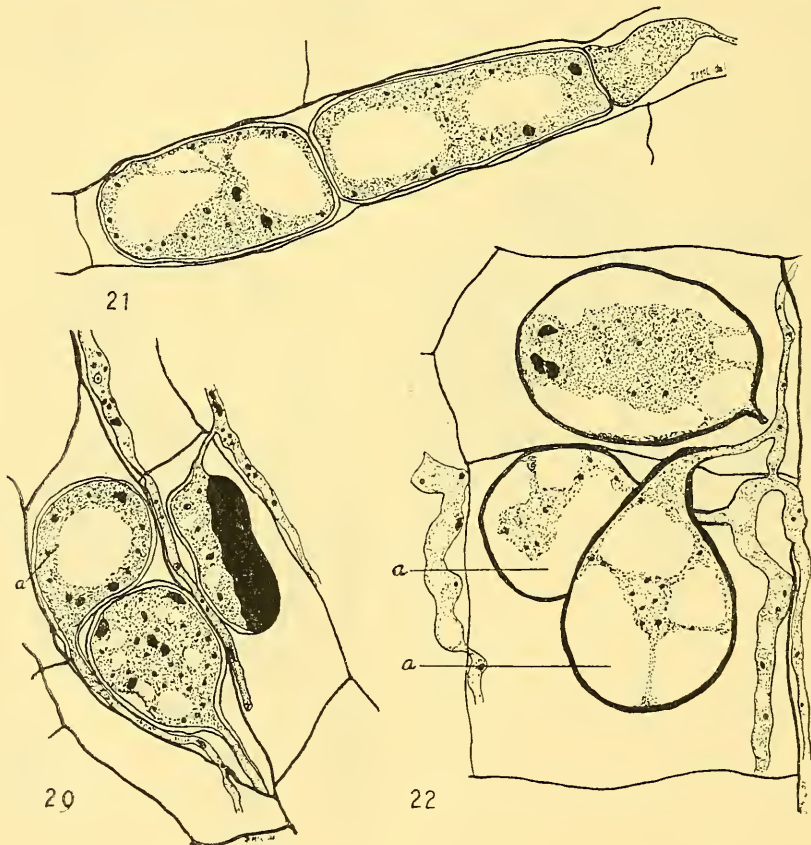
Gradually the cytoplasm of the disrupted sporangioles becomes less and less granular, and the whole mass decreases in bulk, so that by the time small fat globules have aggregated to form the few large globules, only a small quantity remains (Text-figs. 12, 13). The other cell contents at this stage are the nucleus of increased size and chromatin content, a few fragments of collapsed hyphae, and the host cytoplasm associated with the nucleus and the disintegrating endophyte. The hyphae, the remnants of the sporangioles and the fungal cyto-



Text-fig. 18.—Fat accumulation in a complex of sporangioles, and a terminal vesicle (*v*) showing the thick wall; the numerous nuclei (*n*) and irregular fat masses (*f*) are depicted. The vesicle is developed within and distends the intercellular space (*i.sp.*), thus compressing the cortical cells around it. $\times 735$.

Text-fig. 19.—An intercalary vesicle (*a*) developed within an intercellular space. The hypha grows onwards from the apex at (*a*). Fat masses are seen in the vesicle, and in the hyphae. A spherical vesicle is somewhat exceptional in *Eriostemon mycorrhiza*. $\times 735$.

plasm gradually lose their identity and disappear into a dense mass which generally loses all semblance of structure, but is occasionally reticulate (Text-fig. 14): it stains deeply, especially with haematoxylin, and is chemically different from the fat globules which still stain black with the osmic acid. Changes then begin to appear in the large fat globules; lighter areas suggesting vacuolation appear in them when stained with the osmic acid, indicating a change in the reaction from osmic-positive to osmic-negative (Text-figs. 16, 16*a*). As the osmic acid-positive reaction is typical of true fats and lipoids (Cramer, in Lee, 1921), it seems that, owing to the change in the large globules, their chemical nature gradually alters and they lose the power of being oxidized by osmic acid. In all stages of their history these globules are soluble in chloroform and readily stain with Malachite green. Although in their later phase, apparently associated



Text-fig. 20.—A complex of three vesicles, two being terminal and developed within an air-space, and one being formed on a lateral branch of a hypha within a cortical cell. At (*a*) vacuolation is apparent. $\times 735$.

Text-fig. 21.—A linear series of 3 vesicles developed within a single cortical cell. The shape of these vesicles conforms to the cell lumen. $\times 735$.

Text-fig. 22.—A group of 3 vesicles, all formed within cortical cells on branches of the same intercellular hypha. At (*a*) advanced vacuolation is seen. Gradually the vesicles are reduced to empty sacs. $\times 735$.

with their digestion and removal from the cell, they do not react like typical fats and lipoids, they nevertheless possess the properties of fatty substances.

While they are osmic-positive they also stain pink with Sudan III in glycerine when fresh sections are treated, but in their final stage when they are partly or wholly osmic-negative they stain yellow with this reagent.

From these microchemical reactions it seems that true fats are present in the hyphae, arbuscules, sporangioles and within the cell cavity after disruption of the sporangioles, and even until the hyphal complex and cytoplasm of the sporangioles are well on the way to digestion. Their positive reaction is gradually lost owing to their removal from the cells when they are probably no longer true fats. Frequently, after the removal of the large fat globules from the host cell in which they were accumulated, there is left a mere shell which stains dark with osmic acid (Text-fig. 16a).

The large globules of fatty material which accumulate in the cells of the host root, owing to liberation from the ruptured sporangioles, are ultimately removed, presumably by the host. Similar globules have been observed occasionally in the epidermis and frequently in cells of the outermost cortical layer (Text-fig. 17). They are often so large that they distend the cell-membranes. As these cells do not show any trace of the presence of arbuscules, of disintegrating sporangioles, or of hyphae, it must be assumed that they have been translocated there from other cells in which they had been previously accumulated.

This observation indicates movement of fatty substances from infected to non-infected cells of the root, and their re-formation in a similar chemical form. Why fats should be accumulated in the superficial layers of the root, rather than entirely in the inner layers next the stele, is a problem not readily solved.

We have observed no trace of fat-accumulation in the phloem of the stele, when sections are made from material fixed in strong Flemming's solution; therefore, if these substances are removed from the cortex of the root and are employed in growth or flowering of the plant, they are not represented within the stelar tissues as osmic-positive fatty substances, but as some more readily diffusible osmic-negative material, the identity of which we have not determined.

In addition to the osmic acid-positive fat globules there also occur other globules (probably oil) throughout the cortex; these are generally much smaller than the large fat globules, and do not react to the osmic acid. They stain readily with Malachite green. Their distribution is indefinite and they represent another form of oil or fat either formed by the fungus or by the host. They probably represent a secondary form of the fatty substances formerly liberated from the sporangioles.

(e). *Vesicles.*

(i). *Development* (Text-figures 18, 19, 20, 21, 22).—These structures are common in some roots, especially in the outer half of the cortical region; we have seen as many as twelve in a single longitudinal section of a 3mm. portion of a root. They are developed most commonly within the intercellular spaces and only rarely within the cell cavity. They are large ovoid, occasionally spherical, bodies averaging 68μ in length and 53μ in breadth. Typically terminal on an intercellular hypha, they occasionally develop in an intercalary position, the hypha swelling up to form the vesicle, and then budding out at the apex to continue the growth of the hypha again (Text-fig. 19). Their development within the intercellular spaces causes an encroachment on the surrounding cells and the walls in consequence are generally distorted by their growth pressure

(Text-figs. 18, 19). Vesicles also develop within the cell cavity when they conform generally to the shape of the cell (Text-fig. 21). They may occur in groups of 2 or 3, within the cells or in the intercellular spaces, arising terminally upon a main hypha or upon lateral branches (Text-figs. 20, 21, 22). In their incipient stage the wall is thin and they are filled with dense granular cytoplasm containing a few nuclei and a number of small fatty globules (osmic acid-positive). As the vesicle grows to maturity the nuclei increase in number, either by transfer from the hyphae or by division, and vacuoles appear in the cytoplasm. The cytoplasm frequently aggregates at the base and apex of the vesicles, and the wall becomes thickened when the vesicle develops to its full size. This appears to be the complete development in the living roots of *Eriostemon*, for degeneration then ensues. The vacuolation proceeds, with the removal of the fatty materials, the cytoplasm and nuclei, and finally only the thick wall remains about the empty and collapsing vesicle (Text-fig. 22, a).

(ii). *Function*.—The vesicles have been variously interpreted as vegetative, reproductive or storage structures. Gallaud (1905) regarded the vesicle as a normal phenomenon of all endotrophic mycorrhizas except those of the Orchidaceae, and considered them to be storage organs. The consensus of opinion, however, supports the view that the vesicles are reproductive organs of the fungus which have become more or less aborted within the host tissue. This view receives some support from Peyronel's (1923) observation that vesicles are formed in large numbers in the dead roots of *Triticum sativum*, *Zea Mais*, etc., in the soil, many of them "remplis de spores à tous les degrés de maturation". In consequence he regards them as sporangia, and records a peculiar modification of their outer wall, especially at their apex, to form numerous "canalicules" and fine "crévasses" which render it very fragile. His figures show vesicles with spores within the dead roots of Maize. He comments on the striking resemblance between the endophyte and the Phycmycetes and on the morphological similarity of the vesicles to the sporangia of *Endogone macrocarpa* figured by Tulasne. Our studies of the vesicles of the endophyte of *Eriostemon Crowei* confirm the striking resemblance to *Endogone* species studied by Thaxter (1922).

McLennan (1926) records in some of the vesicles of *Lolium temulentum* numerous spherical bodies, each of which appears to be nucleated and suggests that the vesicles are sporangia containing spores. This occurrence of spore-like bodies in the vesicles within the living tissues of *Lolium* seems to be exceptional, as their development is generally arrested. As the endophyte is most commonly found on young roots, many of which die, it is possible that the maturation of the vesicle normally occurs when the fungus is living a saprophytic existence in the soil or in dead roots.

The vesicles have also been interpreted as cysts which are liberated into the soil on the death and disintegration of the root, and which give rise to a new mycelium which is capable of infecting roots.

It must be admitted that the general morphology of the vesicles and the fact that they become filled with food reserves, develop in the outer cortex, and at times produce spore-like bodies, are in harmony with a reproductive function; but it must be remembered that as yet there has been no experimental confirmation of this function.

In *Eriostemon* no trace of spore-like bodies has been observed in the vesicles of the living roots of the host, and the vesicles examined all seem to pass through the same development, culminating in the withdrawal of the contents and collapse. It would seem that the abortion of these bodies is normally produced by the

physiological interaction of the host and probably through the attenuation of the mycelial strain forming the mycorrhiza—so that they become nothing more than storage organs for the temporary accumulation of food reserves which are later transferred to the host. However, it may be possible to isolate vesicles from dead root-tissues of *Eriostemon Crowei*; and to test their functional capacity to form a mycelium directly or after producing a mass of "spores".

(f). *Physiology of the Mycorrhiza.*

The physiological relationship between the components of the mycorrhizal associations observed in the various phyla of the plant kingdom has naturally attracted the attention of investigators, and numerous hypotheses have been formulated to account for the associations. It is to be expected that many differences of interpretation of the relationships should appear, because, although all endotrophic mycorrhizas fall into two different categories, nevertheless differences in the reactions between the two components of the mycorrhiza may exist.

The various mycotrophic hypotheses seem to fall into the two following categories: (a) parasitism, and (b) mutualism. According to the former hypothesis, the endophyte is a parasite within the cells of the host's roots. According to Bernard's views (1904) the relationship of the endophyte and host, in the Orchidaceae at any rate, is one of chronic parasitism which persists from the time of seed germination to the adult condition, a "maladie parasitaire chronique qui commence à la germination et persiste en général jusqu'à l'état adulte; maladie bénigne", and a natural state for this family. He postulates the maintenance of a remarkable balance between the fungus and the orchid plant; a reciprocal relationship which appears to him to border on disease. The digesting cells of the root were compared with the phagocytes of the animal body, and the fungus was kept in check by the process of digestion comparable to phagocytosis.

Gallaud (1905) also regarded the relation of endophyte to host as parasitism. The fungus living an independent existence within the host and deriving its nutriment from the host cells. In coming to this conclusion he was impressed by the scarcity of hyphal connections between the internal cells of the roots containing the fungus and the external mycelium.

McDougall (1899) believed that the endophyte obtained all or a large amount of its nutriment from the cells of the root, although, as only a small percentage of host-cells are infected by the fungus and its digestion takes place, the host may receive some advantage. He regarded the endophyte essentially as an internal parasite, but owing to the digestion probably giving some benefit to the higher plant, looked upon this association as symbiotic in which definite benefit accrued to the higher plant at least.

The latter hypothesis of mutualism or symbiosis, has probably the greatest number of exponents. Frank (1885) believed that the endotrophic fungus functioned in the place of root hairs, and provided the plant with mineral salts and organic matter and nitrogenous materials from the humus; in return, the host supplied carbonaceous materials to the endophyte. This hypothesis was later somewhat modified to the effect that the higher plant secured nitrogenous food by the digestion of the fungus in certain cells.

Stahl (1900) hypothecated the absorption of mineral salts by the endophyte, and endeavoured to establish a causal relation between low transpiration and the mycotrophic habit, and high transpiration and the absence of the mycotrophic condition.

Kusano (1911) regards the fundamental relationship as an exchange of nitrogenous material from the fungus to the host. Some indirect support of this view has been provided by Weevers (1916) who, studying the distribution of ammonium salts in mycorrhizal plants and Leguminous tubercles, found that they were lacking or present in very small amounts in the former, and concluded that nitrogen fixation, if facilitated by the root fungus, must be brought about in a different way from that in the Leguminosae; that the mycotrophic plants with the assistance of the fungus are able to use organic nitrogenous compounds from the soil.

The nitrogenous hypothesis has been supported by numerous investigators, often without any experimental evidence; but direct evidence of nitrogen fixation by endophytic fungi isolated from the roots of higher plants has been provided by Ternetz (1907) and Neilson-Jones and Smith (1928). Ternetz succeeded in isolating eight pycnidia-producing forms from the roots of the Ericaceae referable to the genus *Phoma*, and found that their capacity for nitrogen fixation was variously developed. Duggar and Davis (1916) in similar experiments with a *Phoma betae*, isolated from soil and grown on a medium containing a known amount of nitrogen, found a degree of nitrogen fixation comparable to that of the *Phoma* species studied by Ternetz. The values ranged from 3.022mg. to 7.752mg. per 50 grams of culture fluid in 25 days.

Rayner (1922) isolated from *Calluna* roots a pycnia-bearing fungus which showed similar characters to those isolated by Ternetz from other Ericaceae, and assumed that it was capable of nitrogen fixation. This viewpoint was supported by the fact that pure culture seedlings, inoculated with the endophyte and grown on an agar-medium lacking combined nitrogen, developed as vigorously as controls to which potassium nitrate had been added at the rate of 0.5 grm. per litre. The deduction from this experiment seems reasonable, but Neilson-Jones and Smith, using the strain of *Phoma radices callunae* isolated by Rayner from *Calluna*, and eliminating every conceivable source of error, confirmed experimentally Rayner's assumption of nitrogen fixation, securing values approximating to those of Ternetz for other species or strains of *Phoma* from other members of the Ericaceae.

Therefore, so far as the family Ericaceae is concerned, the nitrogen fixation hypothesis of the endophytic mycorrhiza appears reasonably established, but the position is different in other mycotrophic plants.

McLennan (1926) provides quite a different interpretation of the physiological relationship between the endophyte and the root cells in *Lolium temulentum*. She has observed that the fat present in the internal mycelium of the endophyte is transferred to the sporangioles within the host cells, and is later discharged into the cell cavity through disruption of their membrane. The fat is subsequently removed from the host cell, and is utilized by the higher symbiont, leaving a structureless refringent residuum formed by the digestion of the cytoplasm and the hyphae of the ruptured sporangioles. From this evidence McLennan concludes that the nutritive exchange is carbonaceous rather than nitrogenous—the fat or oil being used by the higher plant for its nutrition. She further concludes that the fungus apparently derives little or no benefit from the association and that “the green plant is parasitic on the lowly member, being the aggressive partner of the union”.

Fraser (1931) has described an interesting type of mycorrhiza in *Lobelia dentata* in which the infection of the roots is by means of rhizomorphs, and the endophyte is wholly intercellular throughout the cortex and does not develop

arbuscules or sporangioles. Her figures indicated the accumulation of a "large number of small darkly staining droplets of reserve food material" in the endophyte when treated with osmic acid. These droplets are ultimately transferred to the cortical cells of the host and appear in the primordial utricle especially as small droplets similar in staining properties to those of the endophyte. "Concurrently with this change the latex vessels in the phloem come to contain material which stains darkly with osmic acid", the inference being that the translocation of these osmic-acid-positive droplets from the roots is by way of the laticiferous vessels of the phloem.

From the figures and from the fact that the droplets stain "darkly" with the osmic acid, it would appear that they are fatty bodies, probably true fats. If this be so, the endophyte of *Lobelia dentata* accumulates fat, which is subsequently given up to the higher symbiont on the disintegration of the fungus and the nutritive exchange is primarily carbonaceous in character. Physiologically, therefore, the endotrophic mycorrhiza of *Lobelia* seems similar to that of *Lolium* and *Eriostemon Crowei* in regard to the nutritive relations of the symbionts, but morphologically it belongs to an intercellular type without arbuscules or sporangioles and producing rhizomorphic infection strands.

Our study of the endophytic fungus of *Eriostemon Crowei* has revealed a striking parallelism between *Lolium* and *Eriostemon* in the relationship of endophyte to higher plant. This homoplasmy is all the more striking when we realize that these mycotrophic plants belong to families so remote from each other systematically and morphologically as the Gramineae and the Rutaceae. As is to be expected, there are differences in detail but the same fundamental development in the physiological relations of the symbionts is apparent. There is an accumulation of fats in the intercellular hyphae of the cortical region of the root; these fats react positively to the osmic acid; their origin is a matter for speculation, but probably they are synthesized by the active hyphae of the endophyte from organic materials derived either from the host or from the humus of the soil, or from both sources. These fats are transferred to the developing arbuscules, which are later transformed into sporangioles, and accumulate in numerous small globules. They still retain their positive reaction to osmic acid. Subsequently changes take place in the globules, which lose their true fat properties (as indicated by failure to stain with osmic acid) and disappear from the host cell. A residuum of the disintegrated fungal cytoplasm and hyphal complex of the sporangioles remains within the host cell, often associated with an enlarged nucleus of the latter and its cytoplasm. There is no doubt that in the mycotrophic *Eriostemon Crowei* the fatty substances accumulate within the various parts of the endophyte, are given up to the host cell and ultimately disappear from it, presumably being used by the plant. These processes and nutritive exchange proceed during the life of the rootlets of the host, the endophyte in the young or recently infected rootlets developing to the sporangiole condition, while in the older rootlets it is giving up its nutriment to the plant. We have not determined any definite synchronization between fat liberation into the host cells and flowering and fruiting of the higher plant. All the processes of development of the endophyte are as readily seen in the minute rootlets of tiny seedlings a few inches high as in those of a mature plant in full flower or fruit.

We are not, therefore, in a position to state that the function of the endophyte in *Eriostemon Crowei* is to supply necessary carbonaceous food for the higher symbiont during flowering and fruiting as seems to be possible for *Lolium*. As

Eriostemon is an apparently normal chlorophyll-bearing plant capable of synthesizing its own supplies of carbonaceous substances like any non-mycotrophic plant, the fats which are produced by the fungus and removed by the host subsequent to the disruption of the sporangioles would appear to us to represent merely a subsidiary supply, although fairly considerable. We cannot believe that the supply of fats by the fungus would be adequate for flowering and fruiting of the host. We are inclined to regard the presence of the endophyte, in this instance, as a form of incipient or benign parasitism, in which the host is able to dominate the development of the fungus, and finally to bring about the digestion of the intracellular portions of the fungus, namely, the arbuscules and sporangioles. In short the initial parasitism by the endophyte is transformed into parasitism by the host.

In its early stages the endophyte is probably parasitic, for the superficial and epidermal hyphae contain much less cytoplasm and nutritive material than the cortical hyphae, while some of the hyphae in the epidermis are almost empty. The rapid growth and migration of the endophyte, therefore, seem to depend principally on the absorption of food from the cells of the host, the excess of food from the mycelium being stored in the cytoplasm as osmic-positive fats. However, the numerous connections between the internal and external hyphae probably provide organic material for the fungal synthesis, and subsequent growth, and water and inorganic salts for the host, as it does not seem probable that the whole of the water and mineral requirements of the plants are provided by the scanty root hair system.

The arbuscules probably represent haustoria for the more efficient absorption of food supplies from the cells of the host, and the subsequent development of the sporangioles and fat accumulation in them are caused by some chemical or physiological action of the host cytoplasm upon them. It is chiefly at the time of sporangiole development that the dominant effect of the host becomes apparent; for, up to the time of arbuscule formation, the behaviour and growth of the fungus are compatible with parasitism. From the beginning of sporangiole development, the relation of host and endophyte becomes changed but the cause of this change can only be surmised.

Judging from its morphological characters, especially the structure of the vesicles, the endophyte seems to resemble species of the genus *Endogone*, but its isolation (if possible) may assist us to identify it. Isolation work is proceeding, with a view to (a) identification of the fungus, (b) attempts to synthesize the mycorrhiza by inoculation of aseptically raised seedlings, (c) discover whether the mycotrophic condition in *Eriostemon* is obligatory, and (d) test the capacity of the endophyte for fixation of nitrogen.

SUMMARY.

1. It has been demonstrated that *Eriostemon Crowei* F.v.M. and other species and genera of New South Wales Rutaceae are mycotrophic.
2. A detailed cytological examination of the endophyte has been made and the mycorrhiza falls into Gallaud's first series, for the hyphae are both intracellular and intercellular.
3. The surface of the root is reticulated in places with the mycelium of a fungus, which is septate, brown in colour and with thick walls and scanty cell contents. It adheres closely to the epidermal cells, branches frequently and develops swellings resembling appressoria from which the infecting hyphae arise.

4. Root infection takes place through the epidermis directly, and the infecting hypha frequently forms a closely coiled mass within the epidermal cells. These epidermal hyphae are thick walled and generally have sparse contents and poor staining capacity.

5. The migration of the endophyte takes place in a transverse and a longitudinal direction, chiefly along the intercellular spaces, but frequently through the cell-walls or cavities. These cortical hyphae are irregular, thin walled, with abundant granular cytoplasm, numerous nuclei and fat masses. Dimorphism of the hyphae is apparent in the arbuscule-producing endophyte, the epidermal hyphae being quite different in appearance from the cortical hyphae, which alone form the arbuscules and sporangioles.

A second endophyte with moniliform hyphae occurs in the epidermis and cortex of the roots; there is, therefore, double-infection by an arbuscule-forming endophyte and by a *Rhizoctonia* form. A *Rhizoctonia* form has been isolated from roots, but the arbuscule-forming type has not yet been isolated.

6. Arbuscules, both simple and complex, are formed from the intercellular hyphae or from the intracellular hyphae within the cortex. Later the arbuscules develop into groups of sporangioles.

7. Fats which react positively to the osmic acid of Flemming's fluid are present in the intercellular hyphae, in the arbuscules, and in the developing sporangioles. Ultimately the fat accumulates in the sporangioles, and is later liberated into the host cavity on their disruption. Sometimes hundreds of small globules are present in a cell. Later they coalesce to form several very large globules. They still react positively to osmic acid and appear homogeneous.

8. The large fat globules later stain unevenly with osmic acid, and finally do not stain at all. Thus the globules are no longer true fats. At the same time their reaction to Sudan III in glycerin changes from pink to a yellowish-brown colour. They still stain with Malachite green and are still soluble in chloroform. They ultimately disappear from the cell, and are probably used by the host. Large fat globules also appear in uninfected cells of the epidermis.

9. As these processes can be seen in very small seedlings as well as in mature plants in full flower and fruit, we cannot co-ordinate the disappearance of the fats with the period of flowering and fruiting of the higher plant, as McLennan is able to do for *Lolium*.

10. The remnants of the sporangioles and hyphae become disintegrated and reduced to a structureless but occasionally reticulate residuum which remains in the cell.

11. After the liberation of the fat from the sporangioles, definite changes become evident in the host nucleus which increases in volume, and shows very pronounced chromatic increase, but no structural hypertrophy, e.g., lobing, etc.

12. Vesicles are developed chiefly in the intercellular spaces of the outer cortex but occasionally within a cortical cell; they are usually terminal, occasionally intercalary or lateral. They contain a large amount of cytoplasm, numerous fat masses and nuclei. Later they develop a thick wall and become vacuolated, and subsequently lose all their contents to the vegetative hyphae or the sporangioles.

13. At first, fat accumulates in all parts of the fungal body, but whether at the expense of the organic materials of the host cells or through synthesis by the fungus itself, from materials removed from the humus of the soil by the numerous infecting hyphae, we cannot say. Ultimately this fat accumulates in the sporangioles within the cells of the host, is liberated into the cell cavities

and is removed by the host. Thus there is an undoubted exchange of fatty materials from the fungus to the host.

14. While the entrance of the fungus to the host root indicates parasitism, the fact remains that the endophyte is controlled and largely digested by the host cells, which receive considerable quantities of fat and, therefore, in the final resort, the higher plant is parasitic upon its endophyte.

Bibliography.

- BERNARD, N., 1904.—Recherches expérimentales sur les Orchidées. *Rev. gén. de Bot.*, 16, 1904, p. 405.
- CRAMER, in LEE, 1921.—The microtometist's Vade-Mecum. J. and A. Churchill, London, 1921, 8th ed.
- DUGGAR and DAVIS, 1916.—Studies in the Physiology of the Fungi. 1. Nitrogen Fixation. *Ann. Missouri Bot. Gard.*, iii, 1916.
- FRANK, B., 1885.—Über die auf Wurzelsymbiose beruhende Ernährung gewisser Bäume durch Pilze. *Ber. deut. Bot. Ges.*, iii, 1885. (Reference to work in Rayner.)
- FRASER, L., 1931.—An Investigation of *Lobelia gibbosa* and *L. dentata*. I. Mycorrhiza, Latex System and General Biology. *Proc. Linn. Soc. N.S.W.*, lvi, part 5, 1931.
- GALLAUD, I., 1905.—Etudes sur les Mycorrhizes endotrophes. *Rev. gén. de Bot.*, xvii, 1905.
- KUSANO, T., 1911.—*Gastrodia elata* and its Symbiotic Association with *Armillaria mellea*. *Journ. Coll. Agric. Tokyo*, iv, 1911.
- MCDUGAL, D. T., 1899.—Symbiotic Saprophytism. *Ann. Bot.*, xiii, 1899.
- MCLENNAN, ETHEL I., 1926.—The Endophytic Fungus of *Lobium*. ii. The Mycorrhiza on the Roots of *Lobium temulentum* L., with a discussion of the physiological relationships of the organism concerned. *Ann. Bot.*, xi, No. clvii, Jan., 1926.
- MOLLBERG, A., 1884.—Untersuchungen über die Pilze in den Wurzeln der Orchideen. *Jenaische Zeitschrift f. Naturwiss.*, 17, 1884, p. 519.
- NEILSON-JONES, W., and LEWELLYN-SMITH, M., 1928.—On the fixation of Atmospheric Nitrogen by *Phoma radiceis callunae* including a new method for investigating Nitrogen fixation by Microorganisms. *Journ. Exp. Biol.*, 6, No. 2, 1928.
- PETRI, 1918-1919.—Sopra una presunta malattia parassitaria del proppo. *Ann. del R. Ist. sup. forest. naz. di Firenze*, 4, 1918-19, p. 97.
- PEYRONEL, B., 1923.—Fructification de l'endophyte à arbuscules et à vésicules des mycorrhizes endotrophes. *Bull. Tri. Soc. Mycol. France*, xxxix, 1923, p. 119.
- , 1924.—Prime ricerche sulla micorize endotrofiche sulla microflora radicecola della fanerogame. *Rivista di Biologia*, 5, 6, 1924, p. 3.
- RAYNER, M. C., 1922.—Nitrogen Fixation in the Ericaceae. *Bot. Gaz.*, lxxiii, 1922, p. 226.
- STAHL, E., 1900.—Der Sinn der Mycorrhizenbildung. *Jahrb. f. wiss. Bot.*, xxxiv, 1900.
- STOUGHTON, R. H., 1930.—Thionin and Orange G. for the differential staining of Bacteria and Fungi in plant tissues. *Ann. App. Biol.*, 17, 1930, 162.
- TERNETZ, C., 1907.—Über die Assimilation des atmosphärischen Stickstoffes durch Pilze. *Jahrb. f. wiss. Bot.*, xlv, 1907, p. 353 (abstract).
- THAXTER, R., 1922.—A Revision of the Endogonaceae. *Proc. Amer. Acad. Arts and Sci.*, 57, 12, 1922, p. 291.
- VAUGHAN, R. E., 1914.—A Method for the Differential Staining of fungus and host. *Mem. Missouri Bot. Gard.*, i, 1914.
- WEEVERS, T., 1916.—Das Vorkommen der Ammoniak und der Ammonsalze in den Pflanzen. *Recueil des Travaux botanique Néerlandais*, 13, 1916 (abstract).